Automated Assessment of Ki-67 Proliferation Index of Canine Mast Tumours Using Computational Deep Learning

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Introduction

Canine cutaneous mast cell tumours (cMCT) are one of the most prevalent skin tumours encountered in veterinary practice, presenting a spectrum of behaviour ranging from indolent to highly aggressive. The prognosis and treatment decisions for these tumours are influenced by various factors, including histological grading [2, 3 & 4]. Grading of cMCT is currently the mainstay for prognostication.

Ki-67 (using the anti-MIB-1 antibody) is a nuclear antigen widely used as a marker of cell proliferation. Quantification of the proportion of immunopositive nuclei (Ki-67Li) produces a score, with higher scores correlating with poorer outcomes [1, 5 & 6]. However, traditional methods of calculating this score rely on manual counting of cells, which is subjective, time-consuming and prone to interpathologist variability.

Objectives

Develop a novel artificial intelligence (AI)-driven model
Validate the model to assess Ki-67 expression in cMCT whole slide images (WSI)
Assess the prognostic significance of Ki-67Li in both dermal and subcutaneous tumours

Methods

Model Training and Validation

We used Aiforia Create® 6.0 to develop and validate an AI model (Figure 1) trained on representative WSIs, acquired at 40x magnification, from archival formalin fixed paraffin-embedded tissue (FFPE) biopsies.

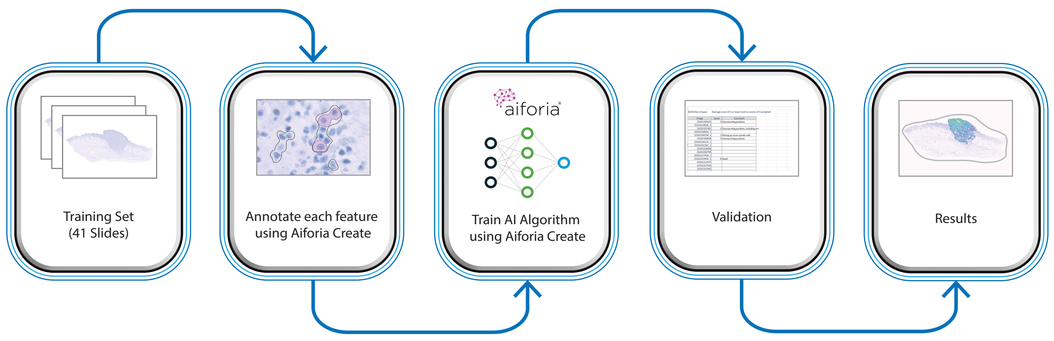

Figure 1. *An overview of the AI training and validation workflow process.*

Select cMCT cases covering a spectrum of grades (41 training, 15 validation)
Perform IHC for Ki-67 (MIB-1 Antibody)
Scan on Philips IntelliSite Ultra Fast digital scanners (x2)
Train AI Model on the WSI – 4 feature layers (Figure 2)
Visual validation by three pathologists, two rounds required (Figure 3)
Validation score <1 = Pass

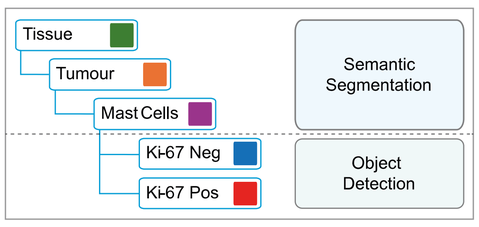

Figure 2. *The AI model structure uses 4 feature layers (Tissue, Tumour and MCT) using semantic segmentation, and Ki-67 positive and negative nuclei, using object detection.*

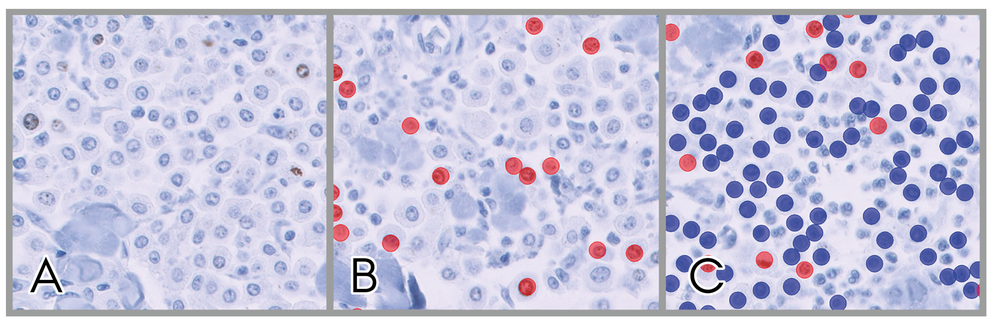

Figure 3. *Ki-67 IHC stained section of a cMCT, during validation, with a haematoxylin counterstain. A high-power view (x40 obj) with object detection of Ki-67 positive and negative nuclei. A) No overlay; B) Postive layer only; C) Positive and negative layers.*

Retrospective, Large Scale Study

191 cMCT cases (previous FFPE) with clinical outcome data
Perform Ki-67 IHC, scan and run AI model
'Hotspot' (Webster et. al [6]) using heatmap (Figure 4) and 'Whole Tumour' % scores
Univariable Cox proportional hazards regression testing (Figure 5)
Establish cutoffs using statistical results
Kaplan-Meier survival estimates calculated (Figure 6)

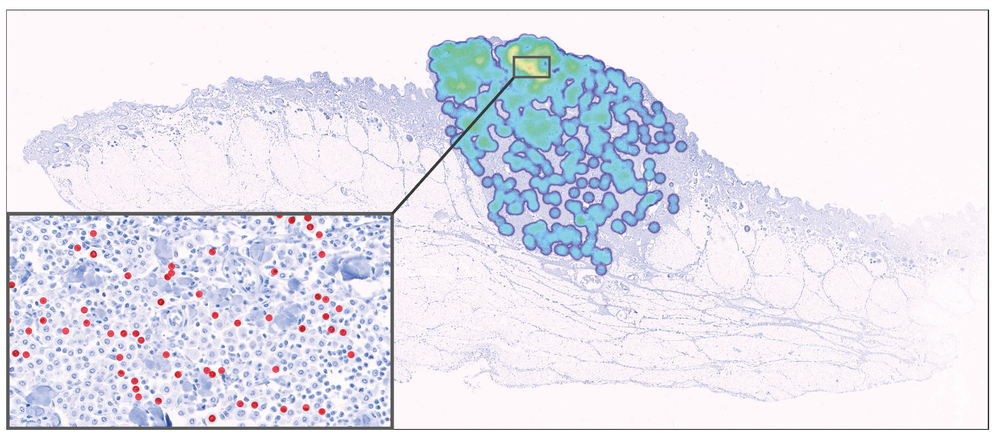

Figure 4. *Model results with heatmap overlay highlights regions with the highest Ki-67 positivity (warmest colours). Inset: A high power view of model results displaying Ki-67 nuclear positivity.*

Results

Significant associations were found with respect to patient outcome with regards to Mast Cell Tumour Specific Survival (MSS) and Mast Cell Tumour Recurrence (MSTR) for both scoring methodologies.

Out of the total 191 dogs with MCTs, where follow-up data was available, 18 (9.1%) died due to their MCT and 20 (10.5%) had recurrence of their MCT; 12 dogs had recurrence of their MCT and died due to their MCT.

Predictive Outcome Summary (Figure 5)

Outcome	Ki-67 Analysis	Group	Number (%)	MAHR (95% CI)	*P*-value
MSS	Hotspot	<= 26.3	121 (68.0%)	-	**0.001**
MSS	Hotspot	>26.3	57 (32.0%)	6.68 (2.13 – 2.01)	
MSS	Tumour % Positive	<=9.57	139 (77.2%)	-	**< 0.001**
MSS	Tumour % Positive	>9.57	41 (22.8%)	7.43 (3.90 – 14.14)	
MSTR	Hotspot	<= 26.3	121 (68.0%)	-	**<0.001**
MSTR	Hotspot	>26.3	57 (32.0%)	9.89 (2.78 – 35.25)	
MSTR	Tumour % Positive	<=9.57	139 (77.2%)	-	**< 0.001**
MSTR	Tumour % Positive	>9.57	41 (22.8%)	5.10 (3.22 – 27.23)	

Figure 5. *Univariable Cox proportional hazards regression reporting the associations of identified optimal cut-offs from AI data for MSS and MSTR (MAHR - MCT attributable Hazard Ratio).*

Hotspot: Ki-67Li cutoff value of 26.3 was highly predictive with respect to animal survival. Patients with a Ki-67Li > 26.3 have a **6.7 times greater** the hazards of dying from all cause mortality as a result of their tumour (MSS). Patients with a Ki-67Li > 26.3 have a a **9.9 times greater** the hazards their tumour will recur after excision (MSTR).

Whole Tumour: A Ki-67Li cutoff value of 9.57% was highly predictive with respect to animal survival. Patients with a Ki-67Li > 9.57% have a **7.4 times greater** the hazards of dying from all cause mortality as a result of their tumour (MSS). Patients with a Ki-67Li > 9.57% have a **5.1 times greater** the hazards their tumour will recur after excision (MSTR).

Predictive Outcome Summary (Figure 6)

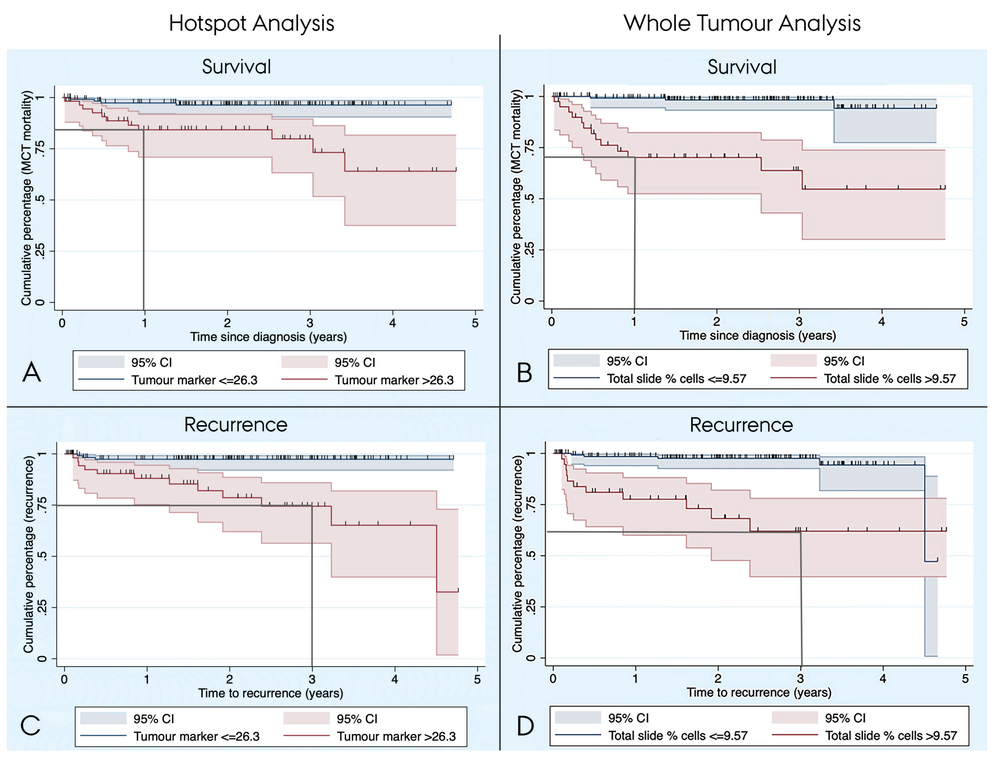

Figure 6. *Kaplan-Meier survival estimate graphs for hotspot and whole slide analysis for MSS and MSTR.*

Hotspot: Within 1 year, **~15% of dogs** with a Ki-67Li >26.3 are predicted to die, due to their cMCT, compared to ~5% of those with Ki-67Li <=26.3, from their cMCT. Within 3 years, **~25% of dogs** with a Ki-67Li >26.3 are predicted to have tumour recurrence, compared to ~5% of those with Ki-67Li <=26.3.

Whole Tumour: Within 1 year, **~29% of dogs** with >9.57% positive cells are predicted to die, due to their cMCT, compared to ~5% of those with <=9.57% positive cells. Within 3 years, **~38% of dogs** with a Ki-67Li >9.57% are predicted to have tumour recurrence, compared to ~5% of those with Ki-67Li <9.57%.

Conclusions

The AI Model achieved comparable results to manual quantification methods in assessing Ki-67Li, showing high accuracy and consistency. It also exhibited a strong correlation with patient outcome (MST and MSTR), indicating its potential in predicting disease progression and patient survival. Both assessment methods, hotspot and whole tumour, demonstrated high specificity and sensitivity.

The hotspot method identified tumours with more typical heterogeneity (foci with high Ki-67 counts), while the whole tumour method provided additional insights for cases with uniformly Ki-67 positive cell counts, indicating a much higher mortality risk in those cases (typically higher grade tumours) above the established cutoff. These findings support the adoption of automated AI-model based Ki-67 scoring for assessing cMCTs.

Acknowledgements

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